THE MORPHOLOGY OF THE ASCOCARP AND SPORE-FORMATION IN THE MANY-SPORED ASCI OF THECOTHEUS PELLETIER I.

JAMES BERTRAM OVERTON.

(WITH PLATES XXIX AND XXX)

ALTHOUGH free cell formation and spore formation have been thoroughly described by several authors, the process has not been followed in asci containing more than eight spores. In view of the old and widely held opinion that the ascus closes a sporophyte generation and is a spore mother cell, the study of the development of a many-spored ascus becomes especially important. The correlative phenomenon of the regular formation of eight nuclei in the ascus by a triple division, whether eight spores or less are to be formed, has been well established; but the variations from the typical method of spore formation and the necessary nuclear and cell divisions, by which more than eight spores are formed, are still in need of further study, and may prove valuable in aiding to solve the character of this peculiar organ. The presence of a true sexual process in the higher fungi, especially among the Ascomycetes, has been established beyond a doubt by the investigations of HARPER and others, but much still remains to be learned concerning the morphology of the sexual organs and ascogonia in the individual forms of this group.

The present investigation was undertaken during the past year, in order to determine the method of spore-formation in many-spored asci. *Thecotheus Pelletieri* presented itself as favorable material. Incidentally stages in the development of the ascocarp have been found which will also be described. The fungus agrees in general with the descriptions and figures of *Thecotheus Pelletieri* (Cr.) Boud., except that the asci are less conspicuously exserted than shown in the figures of CROUAN ('57), BOUDIER ('69), PATOUILLARD ('83), and REHM ('96), and the spores are also faintly colored. BOUDIER, to whom I have also sent material, has had the kindness to examine the fungus and has confirmed the determination, with the note, however, that he finds the spores somewhat larger than in the type.

As will be seen from the description below, the form differs from the species of Ryparobius described by BARKER (:03, :04) in having several ascogonia instead of a single one. As it may be found that this is a character of generic significance, I have thought best to follow BOUDIER in including this form under a separate generic name.

The investigation of the Ascomycetes has shown that there are great variations in the morphology and development of both fruit bodies and reproductive organs, and a sharp distinction may be made as to whether the sexual organs, associated with ascocarp formation, occur singly or in groups. Below I have brought together those forms whose fruit bodies develop from a single ascogonium, as contrasted with those whose ascocarps develop from several ascogonia. In strong contrast to these may also be added a third group of apparently apogamous forms, whose fruit bodies develop directly from a cell of the mycelium without the appearance of sexual organs.

I. In the following forms the ascocarps develop in connection with a single set of sexual organs: Monascus (BARKER :03, OLIVE :05), Dipodascus (JUEL :02), Gymnoascus (BARANETZKY '72, VAN TIEGHEM '76, '77, EIDAM '83, Miss DALE :03), *Erysiphe cichoracearum* (*Sphaerotheca castagnei*) (DEBARY '63, HARPER '95), *Erysiphe galeopsidis* (DEBARY '70), *Erysiphe communis* (DEBARY '70, HARPER '96), *Sphaerotheca humuli* (BLACKMAN & FRASER :05), *Phyllactinia corylea* (HARPER :05), perhaps *Eurotium repens*, *Aspergillus glaucus*, (DEBARY '70), *Penicillium glaucum* (BREFELD '74), *Aspergillus glaucus* (VAN TIEGHEM '77), Chaetomium (VAN TIEGHEM '75, '76, EIDAM '83, ZUKAL '86, OLTMANNS '87), *Stictosphaeria Hoffmanni*, with several species of Diatrype, Eutypa, Quaternaria, and Xylaria (FUISTING '67), *Sphaeria lemaneae*, *Sordaria fimiseda* (WORONIN '70, GILKENET '74), and *Ceratostoma brevirostre*, *Hypocopra* sp. (Miss NICHOLS '96). Among the Discomycetes the following investigated forms show a single ascogonium: *Ascobolus furfuraceus* (JANCZEWSKI '72, HARPER '96), *A. pulcherrimus* (WORONIN '66), *Ryparobius* sp. (BARKER :03, :04), *Peziza granulosa*, *Lachnea scutellata* (WORONIN '66), *Humaria granulata* (BLACKMAN & FRASER :06), *Ascodesmis nigricans* (VAN TIEGHEM '76), and *Thelebolus stercoreus* (RAMLOW :06).

II. *Pyronema confluens* (TULASNE '66, DEBARY '63, KIHLMAN '83, HARPER :00), several species of Collema (BAUR '98), *Parmelia*

acetabulum (BAUR :01, :04), *Anaptychia ciliaris*, *Lecanora subfusca*, *Endocarpon miniatum*, *Gyrophora cylindrica*, *Cladonia pyxidata* (BAUR :04), *Pertusaria communis*, *Pyrenula nitida* (BAUR :01), and *Boudiera Clausseni* (CLAUSSEN :05) represent the only forms thus far described in which the ascocarp is developed in connection with several ascogonia. As described below, Thecotheus also possesses a compound ascogonial apparatus from which the fructification originates.

III. In certain Pyrenomycetes ascocarp formation is apparently independent of sexual organs, being initiated by the formation of a parenchymatous mass of tissue, which is formed by the division of a single hyphal cell of the mycelium, as in Teichospora and Teichosporella (Miss NICHOLS '96) and also in Pleospora (BAUKE '77).

From the above list, selecting the best investigated forms which have a simple ascocarp, we find Dipodascus, Thelebolus, Gymnoascus, Sphaerotheca, Erysiphe, Phyllactinia, Ryparobius, Ascobolus, Humaria, and Monascus. Those having a compound apothecium are several species of lichens (in which, according to BAUR, several hundred carpogonia in some cases may be present in a single apothecium), Pyronema, Boudiera, and Thecotheus.

JUEL (:02) has studied the nuclear phenomena in Dipodascus. The sexual organs arise as short outgrowths of two neighboring cells, each gamete containing several nuclei. After the organs fuse, the walls between break down and the nuclei of the antheridium pass into the oogonium, which grows into a single ascus containing a large fusion nucleus and several smaller nuclei. Although JUEL was unable to make out the details of spore delimitation, he claims that the appearance of the cytoplasm indicates that the spores are formed by free cell formation about the descendants of the fusion nucleus, while the remaining nuclei, which did not fuse in the oogonium, lie scattered in the epiplasm. The actual process of nuclear fusion was not observed.

Miss DALE (:03) describes a sexual fusion of gamete cells in *Gymnoascus Reessii* and *G. candidus*. The oogonia and antheridia are of more or less separated origin. BARKER (:03), IKENO (:03), KUYPER (:04, :05), and OLIVE (:05) have investigated Monascus. BARKER'S and OLIVE'S accounts differ somewhat from that of IKENO. These two authors describe the ascogenous hyphae of Monascus

as arising from an oogonium which has been fertilized by an antheridium, thus establishing a sexual process for Monascus. They also hold that the asci arise from the cells of ascogenous hyphae; while IKENO asserts that there are no ascogenous hyphae, but that uninucleate spore mother cells arise by free cell formation, which in turn form spores also by free cell formation. KUYPER denies any fusion of sexual cells in Monascus, mainly confirms IKENO's observations, and regards the spore mother cells as true asci.

Two forms closely related to Thecotheus in that they belong among the Ascobolaceae have been investigated. BARKER (:**03**, :**04**) in two preliminary notes has described the presence of sexual organs in Ryparobius. The development of the ascocarps was observed step by step under the microscope in hanging drop cultures. The archicarp consists of a small coiled oogonium and a slender antheridium arising from the next cell of the mycelium, growing out over the tip of the oogonium, and fusing at the point of contact. Both antheridium and oogonium are uninucleate when first formed, but subsequently nuclear division occurs in each organ. Nuclear fusion probably occurs, constituting a regular fertilization, although this process was not actually observed. Walls are formed, so that the resulting cells are uninucleate with the exception of the penultimate cell of the ascogonium, which is sometimes found to contain two nuclei lying close together. It will be seen that there is great similarity between Ascobolus and Ryparobius as to the morphology of both the ascogonia and the ascocarps. The ascogenous hyphae, however, do not originate from any particular cell of the ascogonium, as has been described for Ascobolus.

CLAUSSEN (:**05**) has also worked out the morphology of the ascocarp of a form which he at first believed to be *Boudiera hyperborea*, but which HENNINGS (:**03**) described as a new species under the name of *B. Clausseni*. CAVARA (:**05**), basing his conclusions upon CLAUSSEN'S figures, descriptions, and culture methods, believes that this fungus is not a new species of Boudiera, as HENNINGS has described, but that it corresponds perfectly to a species of Ascodesmis, which has been described by VAN TIEGHEM ('**76**) and later by ZUKAL ('**86**). VAN TIEGHEM described in detail two species, *A. nigricans* and *A. aurea*. CAVARA believes that the fungus is *A. nigricans*

Van Tiegh., and that it has been found by him ('89) in the neighborhood of Pavia. DANGEARD (:03) has also briefly described the development of *A. nigricans*, which CAVARA fails to note. So far as I am able to judge from the figures and descriptions of these authors, I see no reason for doubting that CLAUSSEN's fungus represents a new species of Boudiera, as described by HENNINGS. CLAUSSEN grew the fungus on cultures and was able to trace the life history from spore to spore. He found that the archicarps consisted of antheridia and oogonia spirally coiled together and borne in groups. As in Pyronema, the apothecium originates from several pairs of gametes. The ascogenous hyphae appear to originate from any or all of the cells of the ascogonium.

The latest work of HARPER (:05), already referred to, traces the formation of the sexual organs and nuclear fusion in *Phyllactinia corylea* in the minutest detail, showing that the ascocarps develop as the result of fertilization.

BLACKMAN and FRASER (:06) point out that the non-cytological investigation of several forms has shown that a normal sexual process is not to be expected in all, and they have therefore undertaken the cytological investigation of *Humaria granulata*, a form in which no antheridium is present. They find that the oogonium develops as a side branch from multinucleate cells of the mycelium, but that no antheridia are formed as described by WORONIN ('66). The archicarp consists of a varied number of cells, the apical cell of which is much swollen and vacuolate, becoming the ascogonium. Investing hyphae arise from the stalk cells but no antheridia are developed. The ascogonial cell contains a number of nuclei probably formed by division of its primary nuclei. These female nuclei fuse in pairs, thus constituting what they regard as a reduced sexual process, similar to what occurs in the development of the aecidium of *Phragmidium violaceum*. This vegetative fusion occurs in the ascogonia of various ages, and at no definite stage as in Pyronema. These fusion nuclei pass into the ascogenous hyphae, and on account of their size are easily distinguished from those of the vegetative hyphae.

RAMLOW (:06) has shown that the archicarp of *Thelebolus stercoreus* arises as a much twisted uninucleate organ from the uninucleate cells of the mycelium. No antheridium is developed, but the nucleus

of the oogonium divides by successive division until eight free nuclei are formed. Cross walls are then formed in such a manner that one cell contains two nuclei. This cell is larger and finally develops a single ascus, as in Sphaerotheca. No reduced sexual fusion, such as described by BLACKMAN and FRASER, was observed, and RAMLOW believes that Thelebolus is strictly apogamous. Although this form has been placed among the Hemiasci by several earlier authors, RAMLOW from his studies believes it to be a member of the Ascobolaceae, as suggested by SCHROETER and REHM.

That a sexual reproduction occurs in the lichens, comparable to that found in the red algae, as first described by STAHL ('77), and confirmed by BORZI ('78) for other species of Collemaceae, has been practically established by the investigations of several later authors. Although KRABBE'S work ('83, '91) on Cladonia, Baeomyces, and Sphyridium would indicate that sexual organs were absent in these forms, yet WAINIO ('90, '97, '98) claims to have found trichogynes in very young podetia of Cladonia, which would show that sexual organs are present. BAUR'S work ('98) on *C. crispum* confirms STAHL'S observations in every detail. He figures and describes carpogonia and trichogynes. His work also shows that fertilization is a necessity to the development of asci. If the carpogonia are not fertilized by a spermatium, they develop vegetative hyphae; while each cell of the carpogonium which has been fertilized develops ascogenous hyphae. The discovery by STAHL and LINDAU ('88) of trichogynes in *Physcia pulverulenta* has been confirmed by DARBISHIRE ('99), who finds a trichogyne and a carpogonium, each cell of which is uninucleate. The cells of this carpogonium become connected so as to form a more or less continuous structure. LINDAU ('88, '89) has also described the presence of trichogynes in several other species of lichens, but denies that they are sexual organs. In a more recent work BAUR (:01) finds that *Parmelia acetabulum*, *Anaptychia ciliaris*, *Pertusaria communis*, and *Pyrenula nidia* have carpogonia. Anaptychia possesses a single carpogonium, while the other forms have the carpogonia in groups, thus making the fruit body a compound apothecium. BAUR'S opinion is that fertilization occurs by means of spermatia combining with the trichogynes. In a still more recent work BAUR (:04) finds the ascogenous hyphae of Parmelia, Anapty-

chia, Endocarpon, Gyrophora, Lecanora, and Cladonia arising from carpogonia similar to those of Collema, so that these forms also probably possess sexual organs. Solorina is probably apogamous, behaving in this respect like *Peltigera peltidea* and Nephromium as described by FÜNFSTÜCK (:**02**), in which trichogynes have disappeared. SCHULTE (:**04**) has studied the structure of several species of Usnea. In *U. longissima* he claims that the asci do not arise from the ascogonia, although several are present. Trichogynes and spermatia could not be found in *U. microcarpa*.

The presence of trichogynes, spermatia, and carpogonia similar to those of the red algae, and also ascogenous hyphae which arise from carpogonia, all indicate the presence of an undoubted functional sexual apparatus in the lichens, although the important phenomena relating to nuclear behavior still remain unsolved.

GUILLIERMOND (:**05**) has discovered a nuclear fusion accompanying cell fusion in certain yeasts which he holds to be sexual. Conjugation of conidia and nuclear fusion take place in the Schizosaccharomycetes and Zygosaccharomyces just before spore formation, while in certain other forms (*S. Ludwigii*, etc.) the same phenomena occur at the time of spore germination. In the Schizosaccharomycetes and Zygosaccharomyces two cells become connected by a conjugation tube in which nuclear fusion occurs. The fusion nucleus divides immediately and the two daughter nuclei separate, one entering each original cell, in which a double division occurs to form the four nuclei of the four so-called ascospores. In certain other yeasts, such as *S. Ludwigii*, projections are put out from contiguous spores in the ascus, fusing to form a canal in which nuclear fusion occurs. A promycelium is developed from this conjugation tube, which buds off conidia. GUILLIERMOND considers the phenomena here presented as the conjugation of isogamous gametes, in the one case before and in the other case after the formation of the asci. In the one case, *S. Ludwigii*, yeast of Johannisberg, and *S. saturnus*, the sporophyte with the double nuclear characters is the ordinary vegetative budding stage of the yeast, while in the other case (Schizosaccharomycetes and Zygosaccharomyces) this vegetative stage is the gametophyte.

The most recent investigations on the Ascomycetes and rusts indicate an undoubted alternation of generations in these groups. It

will certainly be interesting, therefore, if the yeasts can be shown to exhibit like phenomena, but apparently these forms need to be further investigated before alternation of generations can be regarded as firmly established.

From the above résumé it will be seen that the doctrine of the sexuality of the Ascomycetes has steadily advanced since DEBARY'S time, but an immense number of forms remain yet to be investigated as to the initial stages of the ascocarp. While much investigation has centered on the sexual apparatus, the study of the development of the ascospores has by no means been neglected. Within recent years a considerable literature has been developed relating to the cytology of the ascus. We are concerned more especially with the method of spore formation and the phenomena of chromosome reduction, and the literature of this phase of the subject may be summarized as follows:

GJURASIN ('93) first observed mitoses in the ascus of *Peziza vesiculosa*, maintaining that the divisions are karyokinetic and that the prophases and anaphases take place inside the nuclear membrane. He discovered well-marked asters but describes no centrosomes. In the last or third division the spindles are placed at right angles to the length of the ascus. The asters of these nuclei persist for a remarkably long time, and the astral rays, although not connected with the nuclei, are folded back over them.

DANGEARD ('94) in studying very young asci of *Peziza vesiculosa* and *Borrera ciliaris* discovered the two primary nuclei of the ascus, which fuse to form the large ascus nucleus. Four nuclei appear in the recurved tip of a young ascogenous hypha. By means of transverse walls the nuclei are so separated that one remains in the end cell, two in the penultimate cell, and one in the antepenultimate cell. The ascus grows out from the penultimate cell and the two nuclei fuse to form the ascus nucleus. This according to DANGEARD is a true sexual union.

HARPER ('95, '96, '97 '99, :00, :05) has made the most thorough study of the structure and division of the nuclei in a number of Ascomycetes. He discovered and described the rôle of the kinoplasmic fibers in the formation of spores. He first counted the number of chromosomes in several species. In *Pyronema confluens* he also

observed that there are at first two nuclei in the recurved tip of an ascogenous hypha, which divide simultaneously by mitosis, thus forming four. One of each pair of nuclei enters the young ascus, which arises as an outgrowth of the penultimate cell. These two nuclei, which in this case are thus shown to be not sister nuclei, fuse to form the ascus nucleus. In a study of spore formation HARPER determined and fully described the process involved in the delimitation of the spores. Each nucleus forms a beak which is connected with a persistent central body, bearing at its outer end the astral rays. These rays bend backward and downward, finally coming in contact laterally and fusing to form a thin membrane, which continues to grow backward until a focal point is reached, thus completing the process of spore delimitation. This kinoplasmic membrane cuts the spore out of a homogeneous mass of protoplasm. He has described this process in *Erysiphe communis*, *Peziza vesiculosa*, *Ascobolus furfuraceus*, *Lachnea scutellata*, *Pyronema confluens*, and *Phyllactinia corylea*.

BERLESE ('99) has studied spore formation in *Tuber brumale*, concluding that the plasma membrane of the spore is formed from the astral rays. His results on nuclear phenomena agree essentially with those of GJURASIN and HARPER. MAIRE (:03, :04, :05) has described the nuclear divisions and ascus formation in *Galactinia succosa* and several other Ascomycetes, and confirms the method of spore formation as described by HARPER.

GUILLIERMOND (:03, :04, :05) studied several species with especial reference to karyokinetic division, the structure of the epiplasm, and the formation of the asci. He confirms the results of MAIRE on *Galactinia succosa*, finding that the tips of the ascogenous hyphae are not recurved, but that the terminal cells give rise to the asci. Wherever spore delimitation was studied it was found to follow the method described by HARPER.

BARKER (:03, :04) in a preliminary study of Ryparobius, a form whose asci contain more than eight spores, finds that the asci are formed from the penultimate cells of the ascogenous hyphae which contain two nuclei, these subsequently fusing to form the ascus nucleus. He also studied the nuclear divisions and spore formation. Sixty-four free nuclei are formed, which become regularly grouped

in the peripheral, dense, granular protoplasm of the ascus. Other series of divisions usually occur and uninucleate spores are eventually formed. The details of spore formation have not been described by BARKER. The description of spore formation in this many-spored form will be of great value, and BARKER's results will be awaited with interest. He is inclined to believe that the whole process of spore formation is intermediate between typical methods in sporangia and asci.

MAIRE (:**05**) concludes that in *Galactinia succosa*, *Pustularia vesiculosa*, *Rhytisma acerinum*, *Morchella esculenta*, *Anaptychia ciliaris*, and *Peltigera canina* the first division of the ascus nucleus is heterotypical, and that the second division is homeotypical. In the prophases of the first division he finds a well-marked synapsis stage. The asci are formed by two different processes, one of which is characterized by the formation of a hyphal system sympodially branched, each cell of which is a synkaryon containing two nuclei which divide by conjugate division. The binucleate terminal cells of these ascogenous hyphae become the asci. He believes that there is a tendency in the Ascomycetes to form a synkaryophyte analogous to that of the Basidiomycetes. MAIRE, however, has been unable to trace these synkaryons back to their first beginnings, which is highly important. In Galactinia the centrosomes and spindles have an intranuclear origin, while the polar asters have an extranuclear origin, developed independently of the intranuclear part. Nuclear beaks are formed in the process of spore delimitation.

GUILLIERMOND (:**05**) finds that in *Acetabula leucomelas* and in *Galactinia succosa* the ascogenous hyphae form a series of binucleate cells, the end cells of which become the asci. *Peziza catinus* presents still another method of ascus formation, which he holds to be analogous to that in *P. vesiculosa*. The terminal cells of the ascogenous hyphae are uninucleate, while the subterminal cells are binucleate. These binucleate subterminal cells bud out a lateral branch to form the ascus, which grows parallel to the filament, and in which nuclear fusion occurs to form the ascus nucleus. GUILLIERMOND has studied the behavior of the chromatin in the asci of *Pustularia vesiculosa*, *Peziza rutilans*, *P. catinus*, and *Galactinia succosa*, and believes that the first ascus mitosis is heterotypical, and that the first mitosis is preceded by a synapsis stage.

FAULL (:05) in a cytological study particularly of *Hydnobolites* sp., *Neotiella albocincta*, and *Sordaria fimicola* finds also numerous cases in which the ascus does not arise from the penultimate cell of the recurved tip of the ascogenous hypha, as originally described by DANGEARD. Such is invariably the case in only eleven out of the thirty-six species studied. In some species he claims that the asci bud out from the penultimate cells of the ascogenous hyphae, in others from the terminal cells, and in a few cases apparently from any cell. The uninucleate state of the ascus is preceded by a fusion of two nuclei, which may be sister nuclei. The centrosome and asters are extranuclear in origin, while the spindles are intranuclear. Enucleate portions of spores may be cut off, as in Podospora. FAULL'S description of spore formation is particularly interesting, as it differs entirely from that described by HARPER. The spindle fibers elongate, bringing the daughter nuclei to the periphery of the sporeplasm, with their centrosomes in contact with the plasma membrane. The spores are delimited about each nucleus by the differentiation of a hyaline, finely granular protoplasm, which begins at the centrosome and finally entirely encloses the sporeplasm. The plasma membrane is subsequently formed from or in this hyaline area, and concurrently with this a second membrane is formed in contact with the first, lining the cavity in which the spore is to lie. FAULL suggests that the membrane may arise by a cleavage in the limiting area, caused by its growth and differentiation, together with a pull on the part of the nucleus. Both plasma membranes are intimately concerned in laying down the exospore. He can find no evidence that the astral rays fuse to form a membrane which cuts out the sporeplasm. FAULL also favors the view which homologizes the ascus with the zoosporangium of the Oomycetes, as an argument in favor of the origin of the Ascomycetes from the Oomycetes.

FAULL (:06) also concludes that the ascus of the Laboulbeniaceae contains a fusion nucleus which divides by three successive divisions. The process of spore formation is, as he states, essentially the same as he has described for other Ascomycetes.

In *Humaria granulata* BLACKMAN and FRASER (:06) find that the asci are usually developed from the subterminal cells of the recurved tips of the ascogenous hyphae. In two cases they found asci

developed from the terminal cells of these hyphae, as described by MAIRE for *Galactinia succosa*. These authors have not made a detailed study of the nuclear phenomena, but believe that spore formation is essentially the same as described by HARPER.

RAMLOW (:06) finds that the single ascus of *Thelebolus stercoreus* arises directly from the ascogonium, as in Spaerotheca. The two primary ascus nuclei fuse, and the resulting nucleus by successive divisions forms an enormous number of free nuclei. The details of karyokinesis have not been followed, although he satisfied himself that the divisions are indirect. The nuclei are evenly distributed in the ascus. The phenomena of spore formation were not accurately determined, but RAMLOW, basing his conclusions on the appearance of the protoplasm, which does not cleave as in the sporangium of the Phycomycetes, the appearance of an epiplasm, and the position of the nucleus at one end of the young spore, believes that the process of spore formation is by free cell formation, just as in Erysiphe.

THE NUMBER OF CHROMOSOMES RECORDED IN ASCOMYCETES.

Plant	Chromosome number	Observer	Year
Aleuria cerea	8	Guilliermond	1903, 1904, 1905
Anaptychia ciliaris	4	Dangeard	1903
Anaptychia ciliaris	8	Maire	1904, 1905
Anaptychia ciliaris	8	Guilliermond	1905
Ascobolus furfuraceus	8	Harper	1895
Ascobolus furfuraceus	4	Dangeard	1903
Ascodesmis nigricans	4	Dangeard	1903
Endocarpon miniatum	4	Dangeard	1903
Erysiphe communis	8	Harper	1900
Galactinia succosa	4	Maire	1903, 1904, 1905
Galactinia succosa	8	Guilliermond	1905
Hydnobolites sp	4 or 5	Faull	1905
Hypomyces Thiryanus	4	Maire	1905
Morchella esculenta	4	Dangeard	1903
Neotiella albocincta	6 or 7	Faull	1905
Otidea onotica	8	Guilliermond	1903, 1904
Peltigera canina	4	Maire	1904, 1905
Peziza catinus	12	Guilliermond	1903, 1904
Peziza catinus	16	Guilliermond	1905
Peziza rutilans	12	Guilliermond	1903
Peziza rutilans	16	Guilliermond	1904, 1905
Peziza Stevensoniana	8	Harper	1895
Phyllactinia corylea	8	Harper	1905
Pyronema confluens	10	Harper	1900
Pyronema confluens	4	Dangeard	1903
Pustularia vesiculosa	4	Maire	1904, 1905
Pustularia vesiculosa	8	Guilliermond	1905
Rhytisma acerinum	4	Maire	1904, 1905
Ryparobius sp	8 (?)	Barker	1904
Sphaerotheca castagnei	4	Dangeard	1903

The determination of the number of chromosomes in the ascus as well as in other cells is of the highest importance, as it will indicate the real nature of alternation of generations in the higher fungi. Since the chromosome number has been determined by several authors for a number of species of Ascomycetes, it may prove useful to summarize the results. (See the foregoing table.)

That there is considerable difference of opinion as to the chromosome number even in the same species of some Ascomycetes is evident from the above table. In Ascobolus and Pyronema HARPER finds eight and ten respectively; while DANGEARD claims that there are four in both species. GUILLIERMOND and MAIRE have established the number eight for Anaptychia; while DANGEARD claims four for this species also. MAIRE counts four chromosomes in *Galactinia succosa* and *Pustularia vesiculosa*, while GUILLIERMOND claims that there are eight in each of these forms.

From the above résumé it seems perfectly evident that no such hypothesis as that the chromosome number four is general among the Ascomycetes, as DANGEARD imagines, can be maintained. In this group, on the contrary, judging from the above facts, related species may vary in their respective chromosome numbers, just as has been found to be the case in many of the higher plants.

In a recent paper MAIRE (:**05**) criticizes GUILLIERMOND (:**05**) for saying that he maintains that there are probably four chromosomes in all Ascomycetes, but admits that he and DANGEARD have formulated practically parallel hypotheses on this point. DANGEARD (:**03**), however, distinctly refers this hypothesis to MAIRE (Séance de la société mycologique de France tenue à Poitiers en Octobre, 1903), and says "Cette découverte a été faites simultanément et d'une manière indépendante par MAIRE et nous: elle offre, semble-t-il, toutes garanties de certitude." It appears from these facts that MAIRE has the responsibility of having first made the claim made by DANGEARD, but which MAIRE now attributes to him. It is perfectly plain that no basis for such a hypothesis exists, a fact which MAIRE apparently fully recognizes.

Since DANGEARD first described the asci in a number of forms as arising from the cell next the terminal one, several deviations from this type have been reported. That no such regular process of ascus

formation obtains in general in the whole group of Ascomycetes, or even in nearly related genera, is clearly evident from a study of the more recent cytological investigations, especially those of MAIRE, GUILLIERMOND, and FAULL. In the following résumé no pretense of absolute completeness is made.

We may note first those very simple forms which have sometimes been classed together as Hemiasci, though in most cases, as KUYPER shows, the types are plainly not closely related. The genera Ascoidea (POPTA '99), Protomyces (SAPPIN-TROUFFY '97, POPTA '99), and Taphridium (JUEL :02) possess a septate mycelium with multinucleate cells, from which the sporangia arise directly without the intervention of sexual organs. The hyphal cells of Protascus (DANGEARD :03) also give rise directly to the asci. Sexual organs are present in Dipodascus (JUEL :02) and Eremascus (EIDAM '83), and the fertilized oogonium forms a single ascus. Monascus (BARKER :03, OLIVE :05) apparently forms a branched ascogenous hyphal system, each cell of which is able to produce an ascus. The heterogeneity of these forms is evident by the fact that the asci arise directly from an oogonium, from hyphal cells, or from the cells of ascogenous hyphae.

In some yeasts the conidia become transformed immediately after nuclear fusion into so-called asci, as in Schizosaccharomyces and Zygosaccharomyces, while in others the conidium is transformed directly into the ascus and fusion comes later, as in *S. Ludwigii* (GUILLIERMOND :05).

In the Exoasci the binucleate cells of the mycelium are transformed immediately into a single ascus, as in *Exoascus deformans* (DANGEARD '94) and Taphrina (SADEBECK '93, IKENO :01, :03). Among the Gymnoascaceae, *G. Reesii* and *G. candidus* (BARANETZKY '72, EIDAM '83, DALE :03) have their asci arising from the end cells of a series of short ascogenous hyphae. If CLAUSSEN'S (:05) Boudiera is really *Ascodesmis nigricans*, as CAVARA (:05) believes, and if Ascodesmis belongs among the Gymnoascaceae, then we have a form in this group whose asci arise from the penultimate cells of the recurved tips of the ascogenous hyphae.

The Perisporiaceae show considerable variation in the method of ascus formation. The early works of DEBARY and others may be

passed over, as the methods used by them were not sufficiently deli cate to enable them to determine the exact method of ascus formation. The end cells of ascogenous hyphae described by DEBARY for Eurotium, for example, may as well represent subterminal cells. In the mildew, *Erysiphe communis* (HARPER '96), the end cells of the ascogenous hyphae may develop the asci. This is also the case in *Phyllactinia corylea* (HARPER :05), but asci may also be formed as lateral branches of intercalary cells. In *Sphaerotheca castagnei* (HARPER '95) and *S. humuli* (BLACKMAN and FRASER :05) the oogonium develops into an ascogonium of five or six cells, of which the penultimate one grows into a single ascus. In this genus there are no ascogenous hyphae, unless we accept the interpretation of BLACKMAN and FRASER that the ascogonium is a single ascogenous hypha, whose penultimate cell develops an ascus in the manner described by DANGEARD. In *Anixia spadicea* (FAULL :05) the asci spring from any cell of the ascogenous hyphal system.

In the Tuberaceae, *T. melanospermum* (DANGEARD '94, GUILLIERMOND :04) has its asci arising as described by DANGEARD; while *Genea hispidula* (FAULL :05) shows a marked variation from this type. In this form the ascus grows out from a curved terminal cell. FAULL suggests that perhaps the only difference between this and the other type is the lack of a cross wall cutting off the uninucleate hyphal tip.

Among the apogamous Pyrenomycetes, such as *Teichospora trimorpha*, *T. aspera*, *T. nitida*, and *Teichosporella* sp. (Miss NICHOLS '96), a uninucleate ascus arises from a single central cell of the perithecial mass. *Hypomyces Thiryanus* (MAIRE :05) follows the method described by DANGEARD; while in *Podospora ascerina*, *P. setosa*, *Sordaria fimicola*, and *S. humana* (FAULL :05) the asci arise from a curved terminal cell of an ascogenous hypha. In *Phyllachora graminis* (FAULL :05) the ascus arises from a curved cell of the tip without the formation of a uninucleate tip-cell.

The Discomycetes are described as showing the greatest uniformity in the method of ascus formation, which may perhaps be due to the fact that more careful work has been done upon them. All the following forms have their asci formed in the manner described by DANGEARD ('94) for *Peziza vesiculosa*: *Borrera ciliaris*, *Acetabula*

calyx (DANGEARD '94), *Peziza Stevensoniana*, *Ascobolus furfuraceus* (HARPER '95), *Lachnea scutellata*, *Pyronema confluens* (HARPER :00), *Aleuria cerea* (GUILLIERMOND :03, :04), *A. amplissima*, and *A. olivea* (GUILLIERMOND :04), *Peziza catinus*, *P. venosa*, *P. rutilans* (GUILLIERMOND :04), *Ascobolus marginatus*, *Otidea onotica* (GUILLIERMOND :03, :04), *Acetabula vulgaris*, *Pyronema confluens*, *Ciboria echinophila*, *Bulgaria inquinans*, *Guilliermondia saccabaloides* (GUILLIERMOND :04), *Peziza* sp. (FAULL :05), *Ryparobius* sp. (BARKER :03, :04), *Neotiella albocincta*, *Acetabula* sp., *Pseudoplectania* sp., (FAULL :05), *Boudiera Clausseni* (CLAUSSEN :05), *Peziza vesiculosa* (MAIRE :05), *Thelebolus stercoreus* (RAMLOW :06), *Humaria granulata* (BLACKMAN and FRASER :06), *Thecotheus Pelletieri* (OVERTON :06). Several variations from the type described above also occur among the Discomycetes. *Galactinia succosa* (MAIRE :03, :05, GUILLIERMOND: 03, :05), *Acetabula leucomelas* (GUILLIERMOND :03, :04, :05), *Acetabula vulgaris* (MAIRE :03) have their asci arising from binucleate terminal cells. GUILLIERMOND (:03) also found that *Pustularia vesiculosa* occasionally has its asci arising in a like manner. *Peziza catinus* and *P. vesiculosa* (GUILLIERMOND :05) occasionally have the asci arising from the subterminal cells of the ascogenous hyphae whose tips are recurved. *Acetabula acetabulum* (GUILLIERMOND :03) has binucleate cells formed in the curved tips of the ascogenous hyphae which do not form the asci, but give birth to a series of two, three, and four cells, of which the terminal ones produce the asci. In *Discina venosa* (FAULL :05) the ascus arises from a curved terminal cell. In *Urnula craterium* (FAULL :05) the ascus may spring apparently from any cell whatever. In *Humaria granulata* (BLACKMAN and FRASER :06) the ascus occasionally arises from the terminal cell.

A number of the Helvellaceae have been studied, which also show great variation in the mode of ascus formation. *Helvella ephippium* (DANGEARD '94), *Morchella esculenta* (DANGEARD '94), *Helvella sulcata*, *H. elastica*, *H. crispa* (GUILLIERMOND :04), *Morchella esculenta*, *M. conica*, *Helvella astra*, *H. lacunosa*, *H. elastica* (FAULL :05) have their asci formed from the penultimate cells of the recurved tips of the ascogenous hyphae, in the manner described by DANGEARD for Morchella. In *Geoglossum ophioglossoides*, *G. hir-*

sutum, *Geoglossum* sp., *Verpa conica*, *Gyromytra sphaerospora*, *Leptoglossum luteum*, *Leptoglossum* sp., *Mitrula phalloides*, *Leotia lubrica*, *L. chlorocephala* (FAULL :05) the ascus grows out from the terminal cell, and no uninucleate end cell is cut off. In *Verpa bohemica* (FAULL :05) is found the very greatest variability; the asci appearing as outgrowths of a terminal cell, or from a second, third, or even fourth cell from the tip.

The lichens *Anaptychia ciliaris* (DANGEARD :03), and *Peltigera canina* (MAIRE :05) have their asci regularly formed according to DANGEARD'S method; but *Anaptychia ciliaris* (MAIRE :05) apparently develops its asci from the subterminal cells of the ascogenous hyphae, while the tips, according to MAIRE, may continue development. BAUR (:01, :04), although he does not describe the method of ascus formation, figures ascogenous hyphae which are recurved in *Pertusaria communis*.

In the foregoing I have endeavored to group our knowledge of the various methods of ascus formation according to the natural classification of the forms investigated, in order that the significance of the variation described may be more apparent. It is the nuclear history which is theoretically most interesting, and is thus most essential to determine the relative ancestry of each of the nuclei which fuse in the ascus. This has been done for very few forms as yet. In Pyronema, HARPER (:00) has been able to trace the origin of the two primary nuclei of the ascus, in a case in which the tips of the ascogenous hyphae at first contain two nuclei. These divide by simultaneous division. Of the four nuclei thus formed, one lies in the terminal cell, and two, which are not sister nuclei, in the subterminal cell which becomes the ascus.

Thecotheus belongs to that group of the Discomycetes (Ascobolaceae) whose small fruit bodies are nearly always found growing freely on dung. The species in question occurs on horse dung. It was found on cultures which were old and partly dried up in the Botanical Laboratory of the University of Wisconsin. These cultures were kept running, yielding an abundant supply of material during the progress of this investigation. The fungus has since been found free in nature. The apothecia are very small, about 0.5^{mm} in diameter and 0.25^{mm} high, of a white or yellowish color. When

wet they are therefore plainly visible to the naked eye. A distinct thin excipulum, or lateral boundary of vegetative hyphae, surrounds the hymenium (*fig. 3*). This excipulum is densely covered with short, blunt ends of protruding vegetative hyphae, which give a hairy or warty appearance to its surface. The sterile tissue of the secondary mycelium extends outward and downward to form a pseudo-parenchymatous skirt-like structure, which also extends along the substratum beneath the hypothecium. This lower layer becomes adjusted to the irregularities of the substratum, from which numerous hyphae enter the fructification. The equilibrium of the whole fruit body is maintained by means of this much broadened pseudo-parenchymatous base (*fig. 3*).

The apothecia are at first globular or cylindrical, later becoming broadened, discoid, and somewhat biscuit-shaped (*fig. 2*). The asci are roughly cylindrical in form, about $220\times35\mu$, their greatest diameter being near the top. Each ascus contains thirty-two ellipsoidal, slightly colored spores, each about $17.5\times28\mu$, which are discharged through a small terminal pore. This pore is at first covered by a small cap or operculum, bounded by a thickened ring in the wall of the ascus where the cover breaks off (*fig. 16*). The asci develop from the subhymenium successively; each fruit body thus contains asci and spores in all stages of development. Numerous long and slender septate paraphyses, about 360μ long, arising from the hypothecium, are thickly packed among the asci. At the surface each paraphysis has a somewhat swollen protruding tip (*figs. 2, 3*). The mature spore contains a single nucleus in a mass of very densely granular protoplasm (*fig. 15*). The thin, very pale exospore is corrugated on its outer surface, much like many other spores of the Ascobolaceae. There is a passage through this exospore at each end, as well as through the thick hyaline endospore, to form what is apparently a terminal germinal pore. The spores are not shot out of the asci, but appear to be squeezed out by turgor, together with the lateral pressure exerted by the turgor of the neighboring asci and paraphyses. They are to be found as a dust on the surface of the apothecium, often adhering in masses. Possibly in consequence of the smallness of the terminal aperture of the ascus the spores are not projected or discharged violently. This condition is not general

among other forms of the Ascobolaceae, in most of which the spores are projected for some distance through the terminal pore of the ascus.

Modifications of the usual strengths of Flemming's chromacetic osmium fixing fluids were exclusively used in the preparation of the material. Portions of the substratum upon which apothecia grew were removed and dropped into the fluids. Beside the mature fruits many younger stages were thus obtained. The sections were stained with safranin gentian-violet orange-G combinations and also with Heidenhain's iron-alum haematoxylin. The sections were cut 5–10 μ thick.

HARPER ('95, '96, :05) has shown that the ascogonia of the mildews consists of a much curved row of cells, each of which contains a single nucleus, with the exception of the penultimate cell, which contains two or several nuclei, and which forms the ascus in Sphaerotheca, out of which grow the ascogenous hyphae in other forms. BARKER (:03, :04) also observed the same kind of ascogonium in Ryparobius; while CLAUSSEN (:05) finds several such ascogonia in Boudiera. The youngest ascogonium which I have thus far observed in Thecotheus consisted of a row of several cells which were already multinucleate (*fig. 1*). HARPER ('96) figures and describes perforations in the cross walls in the ascogonium of Ascobolus connecting the adjacent cells. One of the cells of the row is larger. It is from this particular cell that the ascogenous hyphae arise, and out of which the nuclei enter the ascogenous hyphae from it and the neighboring cells. As soon as the ascogonia become empty they undergo disintegration. BARKER (:04) finds each cell of the row in Ryparobius at first uninucleate and then binucleate or occasionally quadrinucleate. From the cells of the row the ascogenous hyphae delevop. He does not distinctly say that they develop from any particular cell of the ascogonium. CLAUSSEN (:05) finds that the gametes of Boudiera contain several nuclei, which however fuse in pairs in the oogonium. Several walls are formed, so that the resulting ascogonium consists of a row of several cells from which the ascogenous hyphae arise. Thecotheus agrees with Ryparobius and Boudiera in that the ascogenous hyphae do not arise from any single cell of the ascogonium, but from any or all of them (*figs. 1* and *2*). The youngest ascogonia which I have been able to find were

already multinucleate, some containing as many as a dozen nuclei, each much larger than those of the vegetative cells. *Fig. 1* shows a young fruit body with sections of several ascogonia, each of whose cells are multinucleate. No connections between the adjacent cells could be observed.

Unlike Ryparobius and like Boudiera, Thecotheus has a compound fruit body. *Fig. 1* represents a section of a young ascocarp, in which several ascogonia are present. One ascogonium, composed of several multinucleate cells, is shown, which resembles in shape the ascogonium of Ascobolus. In the same section, surrounded by the same investment of vegetative hyphae, may be seen also sections of several other ascogonia. These ascogonia are always closely interwoven, so that they are cut in different planes. Other views of the same ascogonia appear in adjacent sections of this ascocarp.

Concurrently with the development of ascogenous hyphae, investing vegetative hyphae encircle the ascogonia, the young ascogenous hyphae, and the asci. This condition can be seen in *fig. 2*. Remnants of the ascogonia are still plainly visible, and the branching ascogenous hyphae can be seen to contain several nuclei, each with a single nucleolus. The nuclei of the ascogenous hyphae are from three to four times as large as those of the paraphyses, making them therefore easily distinguishable. Each cell of the vegetative tissue contains several nuclei. I have been unable to find any regular series of binucleate cells.

The developing ascogenous hyphae are profusely branched, pursuing such irregular paths that it is impossible to follow their course for any great distance. Transverse longitudinal and oblique sections appear in the preparations, which are only mere fragments of the whole system (*fig. 2*). From *fig. 2*, however, it is plain that the ascogenous hyphae develop considerably before transverse septa are put in. The nuclei are especially abundant near the tips of the branches. At the time the cell division is complete, the tips of the branches of the ascogenous hyphae are bent backward as they push upward among other branches and paraphyses. The terminal cell is uninucleate, while the subterminal cell is binucleate. The nuclei of these branches can be seen to be considerably larger than those of the ascogonium (*fig. 4*). From these binucleate subterminal cells

the asci develop. So far as I have been able to observe, asci were never developed from the terminal cell, or from the third cell from the tip, although either condition may possibly occur. Thecotheus certainly does not contain a system of ascogenous hyphae, each cell of which is a synkaryon, as described for Galactinia and *Pustularia vesiculosa*.

In Thecotheus the subterminal cell of an ascogenous hypha arches up to form the young ascus. The two nuclei apparently fuse to form the ascus nucleus (*fig. 4*). The young club-shaped ascus is filled with a dense finely granular vacuolated protoplasm, in which are situated numerous deeply staining extranuclear granules, probably the metachromatic granules of GUILLIERMOND (:**03**), which have also been observed and described by other authors. The fusion nucleus greatly enlarges as the ascus grows, thus maintaining a definite nucleo-cytoplasmic relationship, as described by HARPER (:**05**). Within the nucleus chromatic filaments are organized, which give the appearance of a loose spirem. Division stages were not observed, but, so far as I have been able to find, the nuclear structures are essentially the same as have already been many times described.

The young ascus, which elongates rapidly, crowding up into the hymenium, is somewhat broadened at its tip, gradually narrowing toward the base. The protoplasm, packed in the tip, is coarse and granular (*fig. 7*). A spore region is organized about the nucleus. A large region, in which the protoplasm is very foamy, is present both above and below the central denser sporeplasm. The ascus increases still more in size, the denser regions at the apex and about the nucleus becoming still more sharply separated by the large vacuolated space (*fig. 8*). A peripheral layer of denser protoplasm connects the apical and central regions. The distinction of central and apical regions and the two large vacuolated spaces with foamy protoplasm persist throughout the process of spore formation (*figs. 8–12*). The primary ascus nucleus divides rapidly by three successive divisions to form eight free nuclei. During these divisions there is a gradual decrease in the size of the nuclei, as has also been observed in other asci. In *fig. 10* it will be seen that each of the eight nuclei are very small compared with the nuclei represented in *figs. 7* and *8*. From the abundant stages found in the conditions represented in *fig. 10*, I am

inclined to believe that there is a pause before further divisions occur and a growth period of these eight nuclei. Not only do the nuclei increase in size before dividing, but the asci also lengthen very much and the protoplasm becomes still more vacuolated and foamy (*fig. 11*). Perhaps this increase in size of the nuclei is also correlated with the increase in the amount of cytoplasm in the ascus. Eventually each of these eight nuclei divides to give sixteen free nuclei, no spores being yet delimited (*fig. 11*). In *fig. 11* each nucleus is about as large as one of the daughter nuclei in *fig. 8*. These nuclei certainly show a marked increase in size over those of *fig. 10*. It will also be observed that the nuclei are irregularly arranged in the central region of the sporeplasm. Each of these sixteen nuclei undergoes still another division, resulting in thirty-two free nuclei being found in the ascus. No figure representing this thirty-two nucleate stage has been drawn, although the nuclei were seen in the preparations. *Fig. 6* represents a portion of such a stage. The nuclei here are also very much smaller than in *fig. 11*.

In Ryparobius BARKER (:**03**, :**04**) finds that the number and size of the spores vary in different asci. More than two hundred were normally found in a single ascus, but as few as sixteen have been seen. In Ryparobius successive nuclear divisions occur rapidly, until sixty-four free nuclei are formed. These become regularly grouped in a dense granular mass of protoplasm around the periphery of the ascus. Other series of divisions now usually occur, and eventually uninucleate spores are formed. In Thelebolus (RAMLOW :**06**) many nuclei arise in the ascus, about each of which a spore is delimited, as described by HARPER.

In Thecotheus the spores are delimited from the homogeneous central portion of the cytoplasm immediately after the formation of the thirty-two nuclei. So far as I have been able to observe, the entire process of spore delimitation is accomplished by means of the kinoplasmic fibers which form the astral radiations of the central body. The nucleus becomes pointed or beaked, bearing a central body at its outer end, from which the kinoplasmic radiations extend (*fig. 6*). The chromatin lies freely in the nuclear cavity, apparently connected with the central body. The process of spore delimitation is apparently precisely like that described by HARPER for *Erysiphe communis*,

Lachnea scutellata, *Pyronema confluens*, and *Phyllactinia corylea*. As soon as the beak has reached a certain length, which is comparatively short in Thecotheus, these kinoplasmic fibers bend downward and grow backward over the nucleus, fusing laterally to form a continuous plasma membrane, which separates the cytoplasm of the spore from that of the epiplasm. The nuclear beak is withdrawn and a somewhat pointed nucleus remains in the young spore (*fig. 6*), which gradually resumes the spherical shape of a resting nucleus. Although the process of spore delimitation is not easily followed in Thecotheus, I am convinced that it is essentially the same as HARPER has described for other Ascomycetes. I have also had an opportunity to compare my own preparations containing this stage with those of HARPER on Erysiphe and Lachnea, which objects he found most favorable for study. I can see no essential differences in appearances. The nuclear beaks do not have any special orientation or relation to the plasma membrane, as has been figured and described by certain authors for other forms. The beaks may lie at any angle during the process of spore delimitation.

BARKER (:**04**) believes the process of spore formation in the many-spored asci of Ryparobius to be unlike that in the typical ascus. "The protoplasm passes through a series of characteristic changes during the development of the ascus, and the whole process of spore formation seems to be intermediate between typical methods in sporangia and asci." We shall await BARKER'S completed account of the spore formation in this form with great interest. We have seen that the method of spore delimitation in the many-spored asci of Thecotheus is exactly similar to that found in typical eight-spored asci. As noted in another connection, FAULL entirely dissents from the method of spore formation as described by HARPER. I have been unable to discover in Thecotheus the presence of hyaline zones in connection with which cleavage takes place to delimit the sporeplasm from the epiplasm as described by FAULL, and I am certain that no such method of spore formation exists in Thecotheus.

Fig. 6 represents a spore in the process of delimitation as described above. In the same figure a very young spore is also shown, which has its delimiting membrane already formed and the nuclear beak withdrawn. It will be observed that the sporeplasm is nearly like

the surrounding epiplasm, perhaps slightly more dense and granular. No particular granular area is present. It is simply a portion carved out of the homogeneous cytoplasm in which the nucleus is situated by means of a delimiting kinoplasmic membrane. In *figs. 12a–12c* slightly older spores are shown. They are arranged in the form of a hollow cylinder around the wall of the ascus. About five young spores are arranged vertically along the ascus walls in any one plane. *Fig. 12a* represents a median longitudinal section of the ascus; *figs. 12a* and *12b* are slices off the same ascus.

BARKER (:**03**) found that the numerous nuclei in the asci of Ryparobius became arranged in the form of a hollow sphere just beneath the wall of the ascus before spore delimitation. Each ascospore when completely formed, therefore, has one end toward the center and the other toward the ascus wall, the resulting arrangement of the spores thus being radial. In Thecotheus the nuclei, as noted above, are not arranged radially, but in the form of a hollow cylinder about the wall of the ascus, in a denser peripheral layer of the epiplasm. The resulting spores, therefore, have their long axes parallel to the wall of the ascus. In this respect Ryparobius and Thecotheus are essentially different. Due to this peripheral arrangement into a hollow cylinder, the spores are forced to occupy considerable space in the ascus, some being pushed up near the tip. The epiplasm is at this time everywhere much more vacuolated than in earlier stages, and the ascus is exceedingly turgid and swollen (*figs. 12a–12c.*)

In *fig. 12a* the spores can be seen to show no sign of an exospore or endospore. DEBARY ('**63**, '**64**) thought that the exospore was laid down upon the surface of the spore from the epiplasm, which explanation seems to have gained rather widespread acceptance. The exact method of exospore formation needs investigation. FAULL (:**05**) believes that two membranes are formed, one being the plasma-membrane of the spore and another formed concurrently with this, which lines the cavity in which the spore is to lie. These membranes occupy the position of the hyaline zone described above. The spore wall is supposed to be laid down between the membrane bounding the epiplasm and the plasma membrane of the spore. FAULL suggests that both membranes are perhaps active in the formation of the spore coats.

According to my own observations, the process of the formation of the spore coats does not agree with the account of FAULL (:05) *Fig. 13* shows a young spore which has grown somewhat beyond those found in *figs. 12a–12c*. The limiting membrane is not perceptibly thickened, showing in sections as an even unbroken line. Just inside this membrane, however, the endospore is seen to be but slightly differentiated from the sporeplasm, being much less vacuolated but more hyaline and granular. A more or less distinct boundary is present between the endospore and the sporeplasm. Exactly how the endospore is formed I am at present unable to state, unless it is laid down by the original plasma membrane, which has gradually withdrawn, secreting the substance of the spore coats as it recedes. Finally, the endospore becomes still more granular and hyaline as the spore develops (*fig. 14*). The outermost portion of the hyaline granular area constitutes the exospore. In *fig. 14*, which is not so highly magnified as *fig. 13*, the central portion of the sporeplasm is highly vacuolated. The nucleus of the spore lies in the center of this mass of protoplasm. The irregularities on the exospore may be due to fixation. Lines are developed on the surface of the spore, finally producing an irregularly branched system of elevations and ridges much like that found on the spores Ascobolus. *Fig. 15* represents a mature spore. The two germinal pores, one at either end, are present, passing through the spore coats. The mature endospore is very granular and highly refractive. The inner protoplasm, bounded by the plasma membrane, is still uninucleate but densely granular. Smaller vacuoles have entirely disappeared. In some mature spores two large spherical oil drops are present, one at either side of the nucleus, but not regularly so. *Fig. 15* is typical.

As the asci dry out, the walls become thickened and hyaline. *Fig. 16* shows the upper portion of a nearly mature ascus, at the apex of which is the cap or operculum in the process of formation. A thickened ring in the ascus wall is formed below the operculum. The mature spores are probably discharged through this terminal pore by the turgor of the ascus and the lateral pressure of other asci and paraphyses. Although several attempts were made to germinate these spores in various sorts of media, I have thus far been unsuccessful. Perhaps it may be necessary for them to pass through an ali-

mentary tract or to be naturally or artificially partly digested before germination will occur, or perhaps the spores tested may not have been mature.

I have shown that ascogonia are present from which the asci arise, although I have been unable to find the earliest stages of these organs in Thecotheus. Since in the forms in which sexual organs do exist, as described by several investigators, an ascogonium arises as the result of nuclear and cell divisions from a fertilized oogonium, I think it practically certain that such oogonia exist in Thecotheus. Since the ascogonia are in groups, several being present in each young ascocarp, it is also safe to conclude that the fruit bodies arise as the product of multiple sex organs, just as in Pyronema and Boudiera. Thecotheus, therefore, is another example of a form among the Ascobolaceae with a compound apothecium.

In Thecotheus the asci are developed from the penultimate cells of the recurved tips of the ascogenous hyphae, and they are at first binucleate, later becoming uninucleate in the usual manner. There is apparently no tendency here toward the condition described by MAIRE and GUILLIERMOND, wherein a system of binucleate cells are formed, as in Galactinia, corresponding to the synkaryophyte in which a long series of binucleate cells occur, the nuclei finally fusing in the basidium, as in the Basidiomycetes and rusts. MAIRE claims to have found that these binucleate cells of the ascogenous hyphae originate from hyphae of the subhymenium, whose cells are multinucleate. The first cell arising from these subhymenial hyphae contains two nuclei, which divide by a conjugate division, giving rise to a series of branching synkaryophytic hyphae, which eventually form the asci. This branched, ascogenous hyphal system MAIRE compares to the hyphal system which gives rise to the basidia in the Basidiomycetes. Although MAIRE and GUILLIERMOND have found this system of ascogenous hyphae in certain forms, it still remains and is of the highest importance to determine how the ascocarps originate in these forms. If, as HARPER (:**05**) suggests, the condition found in Pyronema, and still more advanced in Galactinia and *Pustularia vesiculosa*, could work back until the egg cell was reached, an apogamous condition might result, such as is now found in the Hymenomycetes (Miss NICHOLS :**04**), and the nuclear fusion in the ascus

might have acquired secondarily a sexual significance. Thecotheus with its ascogonia, and presumably also still earlier oogonia, shows no tendency towards this condition. I have been unable to find binucleate cells either in the paraphyses, in the mycelium, or in any of the vegetative cells of this fungus, and am sure that Thecotheus does not possess anything comparable to the synkarophyte of the Basidiomycetes.

The main problem relating to the asci at present is whether they are merely eight-spored sporangia or spore mother cells corresponding to those of the higher plants, and on this point the method of spore formation in a polysporous ascus should throw much light. As noted previously, I have confined my attention principally to Thecotheus on account of the abundance of this apparently very favorable form. The possibility that such asci might show transitional conditions leading over to those found in the sporangia of the lower fungi is very suggestive, and, as noted above, BARKER believes that in the asci of the nearly related genus Ryparobius he has found such transitional forms, although RAMLOW believes the ascus of Thelebolus shows no such sporangial characters. The distinction between typical sporangia and typical asci seems to be sharply drawn. In the sporangia of Sporodinia and Pilobolus HARPER ('99) has found that spore formation is by a process of progressive cleavage by means of furrows, either from the surface of the protoplasm or from vacuoles of the mother cell. The nuclei during the cleavage are in a resting stage and are not concerned in the process. Thus the formation of an epiplasm is precluded. HARPER has described the process of cell formation in *Synchitrium decipiens*, *Pilobolus crystallinus*, and *Sporodinia grandis;* while SWINGLE (:03) has observed the same process in the sporangia of *Rhizopus nigricans* and *Phycomyces nitens*. HARPER has pointed out that this process is not one of free cell formation in the sense in which the term is used for free cell formation in the ascus, in which the cells lie free in the mother cell included in the so-called epiplasm. He also concludes that these two very divergent methods of cell formation in asci and sporangia make it impossible to assume any close relationship between these two structures, and this difference is thus made an argument against the homology of the sporangium of the Phycomycetes and the ascus of the Ascomycetes.

Although attempts have recently been made to discredit HARPER'S results on the method of free cell formation in the ascus, no very convincing evidence has been brought forward to show that it is more like that in the sporangium. JUEL (:**02**) in his work on Taphridium seems to think that HARPER has placed too much stress on the action of the kinoplasmic fibers as one of the chief distinguishing characteristics of the process, saying: "Vorlaüfig können wir nicht die Rolle des Kinoplasmas bei der Zellbildung zur *nota characteristica* der freien Zellbildung machen, sondern müssen diesen Begriff in der herrkömmlichen Weise auffassen." Apparently JUEL has failed to comprehend the essence of HARPER'S definition.

FAULL (:**05**) does not believe that the methods of spore formation in the ascus and sporangium are so different as to prevent assumption of their homology. He favors the view that homologizes the ascus with the zoosporangium of the Oomycetes, as an argument in favor of the origin of the Ascomycetes from the Oomycetes. The most complete account of cell formation in the zoosporangia of the Oomycetes is that given for Saprolegnia and Achlya, although the behavior of the nuclei has not been thoroughly enough studied.

The earliest workers in the study of spore formation were influenced by their *a priori* views on the cell theory as a whole, and NÄGELI used it to support his doctrine that new cells are regularly formed by so-called free cell formation from old ones. PRINGSHEIM ('**51**) believed that spores were formed by simultaneous and not by successive bipartitions of the protoplasm, being completely bounded off before the appearance of a cellulose wall. BÜSGEN ('**82**) in his description of spore formation in the sporangium of the Phycomycetes believed that cleavage is due to a simultaneous formation of cell plates, which break down, being later formed again to separate the spores. BERTHOLD ('**86**) studied oogonia, but assumed that the process in oogonia and sporangia are alike. The peripheral layer of protoplasm which surrounds the central vacuole forms dense rounded masses about definite centers, which constantly increase in size, protruding gradually into the central vacuole. Finally the masses separate and round up, later swelling up so as to become pressed together and flattened. Finally these masses again round up, forming definite eggs or swarm spores. BERTHOLD claims that the position of the spores is predetermined by

centers of attraction, about which the protoplasmic lining of the walls is collected. The whole process is a form of free cell formation, in which the entire protoplasm is utilized, without involving the formation of a periplasm. He holds, therefore, that the sporangium of the Saprolegniaceae represents an advance over forms in which periplasm is formed. The sporangium is differentiated into central vacuole and peripheral protoplasm, and is perhaps a stratified structure in itself, whose polarity is determined by the position of the nuclei, which in turn influence the position of the spores, as has been pointed out by HARPER ('99).

According to the work of ROTHERT ('88), HARTOG ('88), HUMPHREY ('92), TROW ('95), and DAVIS (:03), the sporangium is multinucleate when cut off, the nuclei lying scattered in the peripheral layer of protoplasm. DAVIS practically confirms the account of the earlier authors. The uninucleate spores originate by means of clefts, which proceed from the central vacuole of the sporangium to the periphery, dividing the protoplasm into polygonal areas. The spores are later formed from these uninucleate areas. There is no mitotic division of the nuclei or cytoplasmic centers (coenocentra) in the zoosporangia.

HUMPHREY ('92) first studied oogenesis in Saprolegnia by means of modern technique, but was soon followed by TROW ('95, '99) and HARTOG ('95, '96, '99). There seems to be a great diversity of opinion as to the behavior of the nuclei, which far exceed the ultimate number used in the formation of eggs. HUMPHREY and HARTOG believe that the nuclei fuse in groups to form the functional nuclei. TROW claims that many nuclei degenerate until the requisite number is reached, which results DAVIS (:03) has confirmed, but differs from TROW in regard to the sexuality of the Saprolegniaceae. The oogonium arises as an enlargement of the end of a hypha, into which passes a dense mass of cytoplasm and nuclei. A central vacuole is formed, with a peripheral layer of protoplasm lining the walls in which lie the nuclei. The nuclei divide once by mitosis. The protoplasm aggregates into masses which form the eggs. The process of separating these masses by means of a series of fusing vacuoles, has been described by DAVIS. He finds that the egg initials are formed about cytoplasmic centers (coenocentra), much as has been described

for certain Peronosporaceae. It seems apparent that, even if we accept FAULL'S description of spore formation in the ascus, the data are quite insufficient to support any view which homologizes the ascus with the zoosporangium or oosporangium of the Oomycetes.

BARKER (:**04**) announces that the protoplasm in the developing ascus of Ryparobius shows a series of changes in spore formation, which appear to be intermediate between typical methods in sporangia and asci. The account is only preliminary and has been referred to above. My own studies on the asci of Thecotheus however, have shown the process of spore formation to be as in other typical asci, namely, by means of the kinoplasmic radiations of the nucleus. Although more than eight spores are formed in the ascus, the process of spore delimitation is that found in a typical ascus. There is absolutely no evidence that the process is in the least similar to spore formation as found in the sporangia of the Oomycetes or in those of the Phycomycetes. The results obtained do not seem to throw the least light on the homology or origin of this peculiar organ. It apparently makes no difference whether less than eight spores are formed or more than eight, the phenomena of spore delimitation are exactly the same as found in typical eight-spored asci.

That a true alternation of generations, comparable to that found in the higher plants, exists among the Ascomycetes, is certainly obvious from the fact that the asci eventually arise as the result of fertilization. DEBARY ('**70**) advanced the opinion that the ascus fruit represents an asexual generation, and WORONIN ('**70**) compared it to the sporogonium of the moss, which idea was farther emphasized by HARPER ('**96**) for Erysiphe. The only essential difference is that the egg is never separated from the parental tissue system, agreeing in this respect with that of the red algae. HARPER also pointed out that the ascus is an analogue of the spore mother cell of the higher plants, and that the triple division corresponds to the double division in the spore mother cells of the higher plants, with a probable consequent chromosome reduction in the ascus. This view is further supported by the recent discoveries of MAIRE (:**05**), HARPER (:**05**), and GUILLIERMOND (:**05**) on the nuclear phenomena in the ascus, by which reduction of the number of chromosomes and a consequent return to the gametophyte generally occur. These authors have found

that the first division of the ascus nucleus is preceded by a well-marked synapsis phase, which the most recent zoological and botanical investigations have shown to be the most characteristic and important phase of the heterotypical division. While MAIRE finds a synapsis similar to that described by STRASBURGER (:04), HARPER and GUILLIERMOND have found the phenomena to be essentially the same as in the pollen mother cells of the flowering plants studied by GRÉGOIRE (:04), BERGHS (:04, :05), ALLEN (:04, :05), ROSENBERG (:05), STRASBURGER (:05), MIYAKI (:05), OVERTON (:05), TISCHLER, (:06), and CARDIFF (:06). HARPER finds permanent central bodies in the nuclei of Phyllactinia, and that the chromosomes are permanently attached to the central body and are thus brought side by side in nuclear fusion. On this ground he concludes that the chromosomes are permanent structures, and that they must be bivalent in the nuclei of the young ascus, due to the earlier fusion of the sexual nuclei. These bivalent chromosomes, he holds, further unite in synapsis to form quadrivalent structures, consisting of four somatic chromosomes arranged side by side, thus accounting for a numerical reduction just as in the higher plants. MAIRE says that the first division of the ascus nucleus is heterotypical, while the second is homeotypical, which opinion GUILLIERMOND also holds. HARPER gives no opinion as to which are the reduction divisions. He has pointed out, however, the universality of the occurrence of the double division, following synapsis in the spore mother cells of all higher plants, as necessary to accomplish chromosome reduction, where the chromosomes are bivalent structures. I might also call attention in this connection to the elimination of the double division in embryo sac mother cells of parthenogenetic angiosperms, discovered by JUEL (:00, :05), MURBECK (:01), OVERTON (:04), and STRASBURGER (:05), where reduction is not completed.

HARPER also points out that the universal triple division occurring in the ascus, no matter how many spores are to be formed, is probably to be associated with a quadrivalent character of the chromosomes in the ascus nucleus. Where one nuclear fusion occurs, as in most plants and animals, a double division is necessary to complete the reduction and to distribute the elements to the daughter nuclei; while when two nuclear fusions occur, as in Ascomycetes, a triple

division and a double reduction is necessary to accomplish the same results. This triple division of the ascus nucleus occurs universally, whether two spores, four spores, or eight spores are to be formed. HARPER has pointed out how fundamental this triple division is, since when only two spores are to be formed, as in Phyllactinia, six nuclei degenerate. In such cases the three divisions constitute a single continuous process. That all these divisions persist, no matter how many spores are to be produced, shows their necessity in the process of reduction.

The work of BLACKMAN (:**04**) and his students (:**06**) and of CHRISTMAN (:**05**) have established an undoubted alternation of generations in the rusts, showing that in these forms the series of binucleate cells originate as a result of fertilization. The gamete nuclei persist throughout the sporophyte generation as independent nuclei, dividing by a conjugate division. In the teleutospore these nuclei fuse, and a synapsis stage occurs, followed by a double division which leads to the formation of the four nuclei of the four sporidia. As HARPER suggests, there is a striking parallelism between the teleutospore and the spore mother cell of the higher plants. He believes we are justified in regarding the first and second divisions in the promycelium as respectively heterotypical and homeotypical. As there is only one nuclear fusion in the life cycle of the rust, a consequent double division occurs in reduction. MAIRE (:**05**), finding in Galactinia that certain of the cells of the ascogenous hyphae which give rise to the asci are binucleate, holds to the conception that these binucleate cells correspond to those of the Basidiomycetes as well as to those of the rusts. There should be a series of binucleate cells in the sporophyte in all these groups, whose nuclei should divide by conjugate division, fusion first taking place in the basidium, in the teleutospore, and in the ascus, each of which would be comparable to the spore mother cells of the higher plants. This explanation does not explain, however, the universal occurrence of the third division, which is so general among the Ascomycetes, and which FAULL has found to occur in the Laboulbeniaceae; nor does it account for the apparently secondary nature of the fusions described by BLACKMAN and CHRISTMAN, as compared to those of the red algae, lichens, mildews, and Pyronema. It is certainly of great importance to know

how the ascocarps of Galactinia, Acetabulum, and Pustularia arise, and whether apogamy or parthenogenesis is associated with the appearance of binucleate cells in the ascogenous hyphae.

The cells of the ascogenous hyphae of Thecotheus are not binucleate, and I am inclined to accept for this form HARPER'S interpretation that the asci are spore mother cells, modified by adaptation as explosive organs and as reservoirs of reserve food supply, in which a merely vegetative fusion has occurred to maintain a definite nucleocytoplasmic relationship. A triple division follows to complete the reduction and distribution of the somatic chromosomes to each of the resulting eight nuclei. The sporophyte would thus include the ascogenous hyphae and the asci up to the time of the reduction division, which initiates the gametophyte generation.

In the typical ascus the nuclei of the eight spores contain the gametophyte number of chromosomes, as would also be true when only two or four spores are formed. When any of these spores germinate, they give rise to true gametophyte structures, usually a septate mycelium, which may reproduce itself asexually by conidia before sexual reproduction, as in Eurotium or Erysiphe. It is well known that many ascospores contain more than one nucleus, and, as FAULL and others have shown, these nuclei are formed by mitotic division of the primary spore nucleus. The spore may be septate or non-septate. The typical ascospore is uninucleate and non-septate. Both the number of nuclei and the number of septa in a spore vary from one to many. Spores which are septate have apparently begun an intrasporal germination, the gametophyte forming considerable embryonic tissue within the old spore wall. Spores which are not septate but multinucleate have also undergone embryonic development, but without cell division.

We perhaps should expect from what we know of other Ascomycetes that in many-spored asci, as in Thecotheus and Ryparobius, spores would be delimited as soon as eight free nuclei were formed. This does not occur in either of these forms, but further nuclear divisions take place before the spores are delimited. A closer analysis, however, shows that we have analogies for these conditions in the behavior of other undoubted spore mother cells. Intrasporal germination may be looked upon as comparable to that which occurs in the

spores of many of the pteridophytes and spermatophytes. The spore not only begins its germination while inside the sporangium, but while it is still inside the mother cell. The ascus is not only to be looked upon as a mother cell, but also as a mother cell which functions directly as a sporangium. The prevalent impression is that the history of the gametophyte begins with the division of the mother cell and ends in the act of fertilization. The ordinary product of the division of the spore mother cell is four spores, or in typical Ascomycetes eight spores. In Lilium the first mitosis of the mother cell is heterotypical, while the second corresponds exactly in all details to the second division where normal tetrads are to be formed. We have here a double division completed inside the mother cell, and consequent germination to form a gametophyte inside the mother cell. It does not seem inconsistent, therefore, to think of a mother cell containing a gametophyte, or that the reduction divisions may not give rise directly to morphological spores. These nuclei are gametophytic in character and can give rise to gametophyte structures in the embryo sac. It is not absolutely essential, therefore, that the double division result in spore formation. JUEL (:**00**) found that in *Carex acuta* the usual double division occurs in pollen mother cells, complete cell plates being formed which are later resorbed, so that four nuclei lie inside the wall of the mother cell, three of which disintegrate, the fourth forming a single functional microspore. In Fuchsia (WILLE '**86**) as many as fourteen microspores have been reported from a single mother cell, while more or less than four have been found in several other forms. STRASBURGER and JUEL have also counted numerous microspores formed from a single mother cell. It would appear, therefore, that the double division is necessary, but that the number of spores ultimately formed is very variable. If in Fuchsia the walls of the microspores were eliminated, a striking resemblance to the sixteen-nucleate stage of Thecotheus would result. The number of spores formed and the time of their formation seems to be very variable, but this does not interfere with our conception of the alternation of generations in the flowering plants. That thirty-two free nuclei are formed in Thecotheus before spore delimitation occurs is therefore no more striking than that tetrads are not formed as a result of the double division in Lilium, or that more than four micro-

spores are formed in certain angiosperms. It may well be that all the conditions mentioned represent mere adaptations.

In the lichens many-celled spores occur, which are at first always uninucleate; for example, those of Endocarpon. The embryo gametophyte is formed in the spore, which continues its growth when conditions become favorable. Each cell, however, of the multinucleate septate spore gives rise to a filament when the spore germinates. Each cell of a septate spore is comparable to a spore of Thecotheus, in which walls have not been formed until the nuclear divisions have been completed. Germination occurs in the one case before spore delimitation, and in the other case after spore delimitation. In either case, after eight nuclei are formed we are dealing with gametophyte structures. It is a matter of indifference when germination occurs, or when spore delimitation takes place, so long as the triple division has occurred. The time of spore formation is a matter of adaptation to conditions, but the essential nature of the process seems to be the same in all genuine members of the group of Ascomycetes so far studied.

SUMMARY.

1. The fruit body of Thecotheus is formed from several ascogonia and is therefore a compound apothecium.

2. The ascogenous hyphae arise from any or all of the cells of the ascogonium, and consequently the cells of the ascogonium are not connected by perforations through which the nuclei pass to enter the ascogenous hyphae.

3. The ascogenous hyphae do not in this case constitute a synkaryophytic system.

4. The asci arise from the subterminal cells of the recurved tips of the ascogenous hyphae, which cells are binucleate.

5. The ascus nucleus is formed by the fusion of these two primary ascus nuclei.

6. The ascus nucleus divides by triple division to form eight free nuclei, each of which after a period of rest and growth undergoes further division until thirty-two free nuclei are formed in the ascus.

7. Spore delimitation follows the process described by HARPER.

8. Each spore is uninucleate from the start, no nuclear divisions or septa being formed.

9. The exospore is laid down not from the epiplasm but by deposition from the outer layer of the sporeplasm.

10. No evidence has been found to support the theory that the ascus is homologous with the sporangia of either the Oomycetes or the Phycomycetes.

11. The formation of the large number of spores is evidently an adaptive phenomenon, and does not interfere with the conception that the ascus is a spore mother cell.

UNIVERSITY OF WISCONSIN,
Madison, Wis.

LITERATURE CITED.

ALLEN, C. E., :**04**, Chromosome reduction in *Lilium canadense*. BOT. GAZETTE **37**:464–471.

———, :**05**, Nuclear division in the pollen mother cells of *Lilium canadense*. Annals of Botany **19**:191–258.

———, :**05**, Das Verhalten der Kernsubstanzen während der Synapsis in den Pollenmutterzellen von *Lilium canadense*. Jahrb. Wiss. Bot. **42**:72–82.

BARANETZKY, J., '**72**, Entwickelungsgeschichte des *Gymnoascus Reessii*. Bot. Zeit. **30**:145–158.

BARKER, P. T. B., :**03**, The morphology and development of the ascocarp in Monascus. Annals of Botany **17**:167–236.

———, :**03**, The development of the ascocarp in Ryparobius. Rept. British A. A. S., Southport, 849–850.

———, :**04**, Further observations on the ascocarp of Ryparobius. Rept. British A. A. S., Cambridge, 825–826.

BAUKE, H., '**77**, Zur Entwickelungsgeschichte der Ascomyceten. Bot. Zeit. **35**:314–326.

BAUR, E., '**98**, Zur Frage nach der Sexualität der Collemaceen. Ber. Deutsch. Bot. Gesells. **16**:363–367.

———, :**01**, Die Anlage und Entwickelung einiger Flechtenapothecien. Flora **88**:319–332.

———, :**04**, Untersuchungen über die Entwickelungsgeschichte der Flechtenapothecien. Bot. Zeit. **62**:21–44.

BERGHS, J., :**04**, :**05**, La formation des chromosomes hétérotypiques dans la sporogénèse végétale. I. Depuis le spirème jusqu'aux chromosomes mûrs. La Cellule **21**:173–188. II. Depuis la sporogonie jusqu'au spirème définitif dans la microsporogénèse de l'*Allium fistulosum*. *Idem* 383–384. III. La microsporogénèse de *Convallaria majalis*. *Idem* **22**:43–49. IV. La microsporogénèse de *Drosera rotundifolia*, *Narthecium ossifragum*, et *Helleborus foetidus*. *Idem* 141–160.

BERLESE, A. N., '99, Studi citologici sui funghi. Riv. Pathol. Veg. **8**: 143–152.

BERTHOLD, G., '86, Studien über Protoplasmamechanik . Leipzig.

BORZI, A., '78, Studii sulla sessualitá degli Ascomicete. Nuovo Giorn. Bot. Ital. **10**: 43–78.

BOUDIER, É., '69, Mémoire sur les Ascobolées. Ann. Sci. Nat. Bot. V. **10**: 191–268.

BLACKMAN, V. H., :04, On the fertilization, alternation of generations, and general cytology of the Uredineae. Annals of Botany **18**: 323–373.

BLACKMAN, V. H., and FRAZER, HELEN C. J., :05, Fertilization in Sphaerotheca. Annals of Botany **19**: 567–569.

———, :06, Further studies on the sexuality of the Uredineae. Annals of Botany **20**: 35–48.

———, :06, On the sexuality and development of the ascocarp of *Humaria granulata*. Proc. Roy. Soc. London B. **77**: 354–368.

BREFELD, O., '74, Untersuchungen aus dem Gesammtgebiete der Mykologie. Botanische Untersuchungen über Schimmelpilze. II. Penicillium. Leipzig.

BÜSGEN, M., '82, Die Entwicklung der Phycomycetensporangien. Jahrb. Wiss. Bot. **13**: 253–285.

CARDIFF, I. D., :06, A study of synapsis and reduction. Bull. Torr. Bot. Club **33**: 271–306

CAVARA, F., '89, Matériaux de mycologie lombarde. Rev. Mycol. **12**: 173–193.

———, :05, Causeries mycologiques. Ann. Mycol. **3**: 362–365.

CHRISTMAN, A. H., :05, Sexual reproduction in the rusts. BOT. GAZETTE **19**: 267–275.

CLAUSSEN, P., :05, Zur Entwickelungeschichte der Ascomyceten. Boudiera. Bot. Zeit. **63**: 1–28.

CROUAN, FRÈRES, '57, Note sur quelques Ascobolus nouveaux et sur une espèce nouvelle de Vibrissea. Ann. Sci. Nat. Bot. IV. **7**: 173–178.

DALE, Miss E., :03, Observations on Gymnoascaceae. Annals of Botany **17**: 571–596.

DANGEARD, P., '94, '95, La reproduction sexuelle des Ascomycètes. Le Botaniste **4**: 21–58.

———, '94, '95, La truffe. Recherches sur son développement, sa structure, sa reproduction sexuelle. *Idem* 63–87.

———, '96, '97, Second mémoire sur la reproduction sexuelle des Ascomycètes. *Idem* **5**: 245–284.

———, :03, Sur le nouveau genre Protascus. *Idem* **9**: 23–25.

———, :03, Sur le genre Ascodesmis. *Idem* 33–35.

———, :03, Nouvelles considérations sur la reproduction sexuelle des champignons supérieurs. *Idem* 35–46.

DARBISHIRE, O. V., '99, Ueber die Apothecienentwicklung der Flechte *Physcia pulverulenta*. Jahrb. Wiss. Bot. **34**: 329–345.

DAVIS, B. M., :03, Oogenesis in Saprolegenia. BOT. GAZETTE **35**: 233–249, 320–349.

DeBary, A., '63, Ueber die Fruchtentwicklung der Ascomyceten. Leipzig.
———, '70, Eurotium, Erysiphe, Cicinnobolus, nebst Bemerkungen über die Geschlechtsorgane der Ascomyceten. Beitr. Morph. u. Phys. der Pilze **3**: 1–95.
Eidam, E., '83, Beitrag zur Kenntniss der Entwicklung der Gymnoasceen. Cohn's Beitr. Biol. Pflanz. **3**: 267–305.
———, '83, Zur Kenntniss der Entwicklung bei den Ascomyceten. *Idem* 377–433.
Faull, J. H., :05, Development of ascus and spore formation in Ascomycetes. Proc. Boston Soc. Nat. Hist. **32**: 77–114.
———, :06, A preliminary note on ascus and spore formation in the Laboulbeniaceae. Science N. S. **23**: 152–153.
Fuisting, W., '67, Zur Entwickelungsgeschichte der Pyrenomyceten. Bot. Zeit. **25**: 177–181, 185–189, 193–198.
Fünfstück, M., :02, Der gegenwartige Stand der Flechtenkunde. Ber. Deutsch. Bot. Gesells. **20**: (62)-(77).
Gilkinet, A., '74, Recherches morphologiques sur les Pyrénomycètes. Bull. Acad. Roy. Belgique II. **37**: —. (pp. 28).
Gjurasin, S., '93, Über die Kernteilung in den Schlauchen von *Peziza vesiculosa*. Ber. Deutsch. Bot. Gesells. **2**: 113–117.
Grégoire, V., :04, Le réduction numérique des chromosomes et les cinèses de maturation. La Cellule **21**: 297–314.
Guilliermond, M. A., :03, Contribution à l'étude de l'épiplasme des Ascomycètes, Compt. Rend. Acad. Sci. Paris **136**: 253–255.
———, :03, Contribution à l'étude cytologique des Ascomycètes. *Idem* 938–939, 1088.
———, :03, Nouvelles recherches sur l'épiplasme des Ascomycètes. *Idem* 1487–1489.
———, :03, Contributions à l'étude de l'épiplasme des Ascomycètes et recherches sur les corpuscules métachromatiques des champignons. Ann. Mycol. **1**: 201–215.
———, :04, Contributions à l'étude de la formation des asques et de l'épiplasme des Ascomycètes. Rev. Gén. Bot. **16**: 1–65.
———, :04, Recherches sur la karyokinèse chez des Ascomycètes. *Idem* 129–143.
———, :04, Sur la karyokinèse de *Peziza rutilans*. Compt. Rend. Soc. Biol. **56**: 412.
———, :05, Sur la nombre des chromosomes chez les Ascomycètes. *Idem* **58**: —.
———, :05, Remarques sur la karyokinèse des Ascomycètes. Ann. Mycol. **3**: 344–361.
———, :05, Recherches sur la germination des spores et la conjugaison chez les levures. Rev. Gén. Bot. **17**: 337–377.
Harper, R. A., '95, Die Entwicklung des Peritheciums bei *Sphaerotheca castagnei*. Ber. Deutsch. Bot. Gesells. **13**: 475–481.
———, '95, Beiträge zur Kenntniss der Kernteilung und Sporenbildung im Ascus. *Idem* (67)–(78).

HARPER, R. A., '96, Ueber das Verhalten der Kerne bei der Fruchtentwicklung einiger Ascomyceten. Jahrb. Wiss. Bot. **29**:655–685.

———, '97, Kernteilung und freie Zellbildung im Ascus. *Idem* **30**:249–284.

———, '99, Cell division in sporangia and asci. Annals of Botany **13**:467–524.

———, :00, Sexual reproduction in *Pyronema confluens* and the morphology of the ascocarp. *Idem* **14**:321–400.

———, :05, Sexual reproduction and the organization of the nucleus in certain mildews. Publ. Carnegie Institution, Washington, no. 37, pp. 92.

HARTOG, M., '88, Recent researches on the Saprolegnieae. A critical abstract of ROTHERT'S results. Annals of Botany **2**:201–216.

———, '95, On the cytology of the vegetative and reproductive organs of the Saprolegnieae. Trans. Royal Irish Acad. **30**:649–708.

———, '96, The cytology of Saprolegnia. Annals of Botany **10**:98–100.

———, '99, The alleged fertilization in the Saprolegnieae. *Idem* **13**:447–459.

HENNINGS, P., :03, Einige deutsche dungbewohnende Ascomyceten. Hedwigia Beibl. **42**:181–185.

HUMPHREY, J. E., '92, Saprolegniaceae of the United States. Trans. Am. Phil. Soc. **17**:63–148.

IKENO, S., :01, Studien über die Sporenbildung bei *Taphrina Johansoni* Sad. Flora **88**:229–237.

———, :03, Die Sporenbildung von Taphrina-Arten. *Idem* **92**:1–31.

———, :03, Ueber die Sporenbildung und systematische Stellung von *Monascus purpureus* Went. Ber. Deutsch. Bot. Gesells. **21**:259–269.

JANCZEWSKI, E,. DEG. '72, Recherches morphologiques sur l'*Ascobolus furfuraceus*. Ann. Sci. Nat. Bot. V. **15**:197–214.

JUEL, H. O., '97, Die Kernteilungen in den Pollenmutterzellen von *Hemerocallis fulva* und die bei denselben auftretenden Unregelmässigkeiten. Jahrb. Wiss. Bot. **30**:205–226.

———, :00, Beiträge zur Kenntniss der Tetradenteilung. *Idem* **35**:626–659.

———, :00, Vergleichende Untersuchungen über typische und parthenogenetische Fortpflanzung bei der Gattung Antennaria. Konigl. Svensk. Vetensk. Akad. Handl. **33**:no. 5. pp. 59.

———, :02, *Taphridium* Lagerh. und Juel. Eine neue Gattung der Protomycetaceen. Konigl. Svensk. Vetensk. Akad. Handl. **27**:no. 16. pp. 29.

———, :02, Ueber Zellinhalt, Befruchtung und Sporenbildung bei Sporenbildung bei Dipodascus. Flora **91**:47–55.

———, :05, Die Tetradenteilungen bei Taraxacum und anderen Chicorieen. Konigl. Svensk. Vetensk. Akad. Handl. **39**:no. 4. pp. 21.

KIHLMAN, O., '83, Zur Entwicklungsgeschichte der Ascomyceten. Acta Soc. Sci. Fenn. **13**:Sonderabdr. 1–43.

KRABBE, G., '83, Morphologie und Entwickelungsgeschichte der Cladoniaceen. Ber. Deutsch. Bot. Gesells. **1**:64–77.

———, '95, Beiträge zur Kenntniss der Ascomyceten. Leipzig.

KUYPER, H. P., :04, Die Perithecium-Entwicklung von *Monascus purpureus* Went. und *Monascus Barkeri* Dang., und die systematische Stellung dieser Pilze. Ext. du Recueil des Travaux Bot. Neer.

———, :05, Die Peritheciumentwickelung von *Monascus purpureus* Went. und *Monascus Barkeri* Dang. so wie die systematische Stellung dieser Pilze. Ann. Mycol. **3**:32–81.

LINDAU, G., '88, Ueber die Anlage und Entwickelung einiger Flechtenapothecien. Flora **62**:451–489.

———, '89, Beiträge zur Kenntniss der Gattung Gyrophora. Festsch. f. Schwendener. Berlin.

MAIRE, R., :03, Recherches cytologiques et taxonomiques sur les Basidiomycètes. Bull. Soc. Mycol. France **18**:1–209.

———, :03, Recherches cytologiques sur les *Galactinia succosa*. Compt. Rend. Acad. Sci. Paris **137**:769–771.

———, :03, La formation des asques chez les Pezizes et l'évolution nucléaire des Ascomycètes. Compt. Rend. Soc. Biol. **55**:1401.

———, :04, Remarques sur la cytologie de quelques Ascomycètes. *Idem* **56**:86.

———, :04, Sur les divisions nucleaires dans l'asque de la morelle et de quelques autres Ascomycètes. *Idem* 822.

———, :05, La mitose hétérotypique chez les Ascomycètes. Compt. Rend. Acad. Sci. Paris **149**:950–952.

———, :05, La mitose hétérotypique et la signification des protochromosomes chez les Basidiomycètes. Compt. Rend. Soc. Biol. **58**:726.

———, :05, Recherches cytologiques sur quelques Ascomycètes. Ann. Mycol. **3**: 123–154.

MIYAKI, K., :05, Ueber Reduktionsteilung in den Pollenmutterzellen einiger Monokotylen. Jahrb. Wiss. Bot. **42**:83–120.

MURBECK, S., :01, Parthenogenetische Embryobildung in der Gattung Alchemilla. Lunds. Univ. Årssk. **36**:no. 7. pp. 41.

NICHOLS, Miss M. A., '96, The morphology and development of certain pyrenomycetous fungi. BOT. GAZETTE **22**:301–328.

NICHOLS, Miss S. P., :04, The nature and origin of the binucleated cells in some Basidiomycetes. Trans. Wis. Acad. Sci. **15**:30–70.

OLIVE, E. W., :05, The morphology of *Monascus purpureus*. BOT. GAZETTE **39**:50–60.

OLTMANNS, F., :87, Ueber die Entwickelung der Perithecien in der Gattung Chaetomium. Bot. Zeit. **45**:196–199, 210–218, 250–254.

OVERTON, J. B., :04, Ueber Parthenogenesis bei *Thalictrum purpurascens*. Ber. Deutsch. Bot. Gesells. **22**:274–283.

———, :05, Über Reduktionsteilung in den Pollenmutterzellen einiger Dicotylen. Jahrb. Wiss. Bot. **42**:121–153.

PATOUILLARD, N., Tabulae analyticae fungorum 1:74. Poligny.

POPTA, M. L., '99, Beitrag zur Kenntniss der Hemiasci. Flora **86**:1–46.

PRINGSHEIM, N., '51, Die Entwickelungsgeschichte der *Achlya prolifera*. Nov. Act. Caes. Leop. Carol. Nat. Cur. **23**:397–460.

RAMLOW, G., '96, Zur Entwicklungsgeschichte von *Thelebolus stercoreus*. Bot. Zeit. **64**:85–99.

REHM, H., '96, Rabenhorst's Kryptogamen-Flora IV. Pilze **3**:1000.

ROSENBERG, O., :05, Zur Kenntniss der Reducktionsteilung im Pflanzen. Bot Not. **1905**: 1–24.

ROTHERT, W., '88, Die Entwickelung der Sporangien bei den Saprolegnieen. Cohn's Beitr. Biol. Pflanzen **5**:291–349.

SADEBECK, R., '93, Die parasitischen Exoasceen. Jahrb. Hamburg. Wiss. Anstalt **10**:no. 2. pp. 110.

———, '95, Einige neue Beobachtungen und Kritische Bemerkungen über die Exoascaceae. Ber. Deutsch. Bot. Gesells. **13**:265–280.

SAPPIN-TROUFFY, '96, '97, Note sur la place du *Protomyces macrosporus* dans la classification. Le Botaniste **5**:285–288.

SCHULTE, F., :05, Zur Anatomie der Fletchtengattung Usnea. Beih. Bot. Centralb. **18**:1–22.

STAHL, E., '77, Beiträge zur Entwickelungsgeschichte der Flechte. I & II. Leipzig.

STRASBURGER, E., '80, Zellbildung und Zellteilung. Jena.

———, :04, Ueber Reduktionsteilung. Sitzungsber. Kön. Preuss. Akad. Wiss. **18**:587–614.

———, :04, Die Apogamie der Eualchemillen und allgemeine Gesichtspunkte, die sich aus ihr geben. Jahrb. Wiss. Bot. **41**:88–164.

———, :05, Typische und allotypische Kernteilung, Ergebnisse und Erörterungen. Jahrb. Wiss. Bot. **42**:1–71.

SWINGLE, D. B., :03, Formation of the spores in the sporangia of *Rhizopus nigricans* and of *Phycomyces nitens*. U. S. Dept. Agr., Bureau Plant Ind., Bull. 37:1–40.

TISCHLER, G., :06, Über die Entwicklung des Pollens und der Tapetenzellen bei Ribes-Hybriden. Jahrb. Wiss. Bot. **42**:545–578.

———, :06, Ueber die Entwicklung der Sexualorgane bei einem sterilen Bryonia-Bastard. Ber. Deutsch. Bot. Gesells. **24**:83–96.

TROW, A. H., '05, The karyology of Saprolegnia. Annals of Botany **9**:609–652.

———, '99, Observations on the biology and cytology of a new variety of *Achlya americana*. Annals of Botany **13**:131–179.

TULASNE, R. & C., '65, Selecta Fungorum Carpologia **3**:197–198.

———, '66, Note sur les phénomènes de copulation que présentent quelques champignons. Ann. Sci. Nat. Bot. V. **6**:211–220.

VAN TIEGHEM, P., '75, Sur le développement du fruit des Chaetomium et la prétendue sexualité des Ascomycètes. Compt. Rend. Acad. Sci. Paris **81**: 1110–1113.

———, '76, Sur le développement du fruit des Ascodesmis. Bull. Soc. Bot. France **23**:271–279.

VAN TIGHEM, P, '76, Nouvelle observations sur le développement du fruit et sur la prétendue sexualité des Basidiomycètes et des Ascomycètes. *Idem* 99–105.

———, '76, Nouvelles observations sur le développement du périthèce des Chaetomium. *Idem* 364–366.

———, '77, Sur le développement de quelques Ascomycètes. *Idem* **24**: 96–105, 159–169, 203–206, 206–210.

WAINO, E. A., '90, Étude sur la classification naturelle et la morphologie des lichens du Brésil. Helsingfors.

———, '97, '98, Monographia Cladoniarum universalis. Acta. Soc. Fauna et Flora Fennica **14**: 1–268.

WILLE, N., '86, Ueber die Entwickelungsgeschichte der Pollenkerne der Angiospermen und das Wachstum der Membranen durch Intussusception. Christiania.

WORONIN, M., '66, Zur Entwickelungsgeschichte des *Ascobotus pulcherrimus* und einiger Pezizeen. Beitr. Morph. u. Phys. Pilze **2**: 1–11.

———, '70, *Sphaeria lemaneae*, *Sordaria carpophila*, *S. fimiseda*, *Arthrobotrys oligospora*. Beitr. Morph. u. Phys. Pilze **3**: 1–36.

ZUKAL, H., '86, Mycologische Untersuchungen. Denk. Kais. Akad. Wiss. Wien **51**: 21–36.

EXPLANATION OF PLATES XXIX AND XXX.

All drawings were made with the aid of a Bausch and Lomb camera lucida, together with a Bausch and Lomb $\frac{1}{12}$ oil-immersion lens. Plate XXX has been reduced one-third in reproduction.

PLATE XXIX.

FIG. 1. Section of young ascocarp showing portions of ascogonia, whose cells are already multinucleate.

FIG. 2. Median section of an older ascocarp showing ascogonia, young ascogenous hyphae, and paraphyses.

FIG. 3. Median vertical section showing structure of a nearly mature ascocarp.

FIG. 4. Section showing young ascogenous hypha; the terminal cell is uninucleate and the sub-terminal cell, which is to form the ascus, is binucleate.

FIG. 5. Young ascus showing fusion nucleus with two nucleoli.

FIG. 6. Portion of an ascus at time of spore delimitation, showing nuclear beak with a system of astral rays; a young spore is also shown, whose plasma membrane is completed.

PLATE XXX.

FIG. 7. Young ascus showing primary fusion nucleus with one nucleolus.

FIG. 8. Ascus with two nuclei.

FIG. 9. Ascus with four nuclei.

FIG. 10. Ascus with eight nuclei.

FIGS. 11a, 11b. Two sections of the same ascus, showing sixteen free nuclei, each of which will undergo another division to form thirty-two free nuclei.

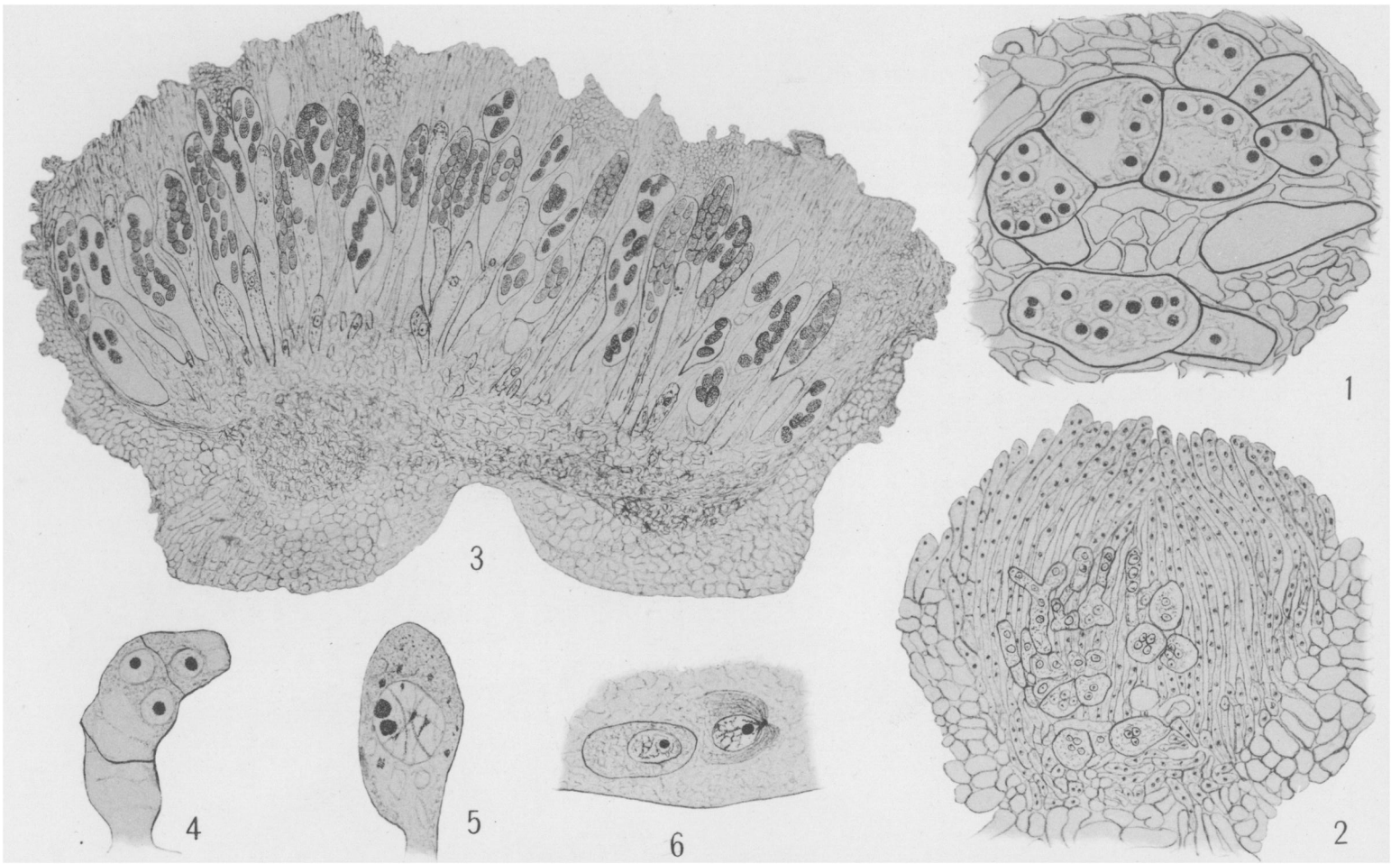
FIGS. 12a, 12b, 12c. Three sections of the same ascus showing thirty-two young spores; *fig. 12a*, which is a median section, shows the arrangement of the spores about the ascus wall.

FIG. 13. Young spore showing early formation of spore wall.

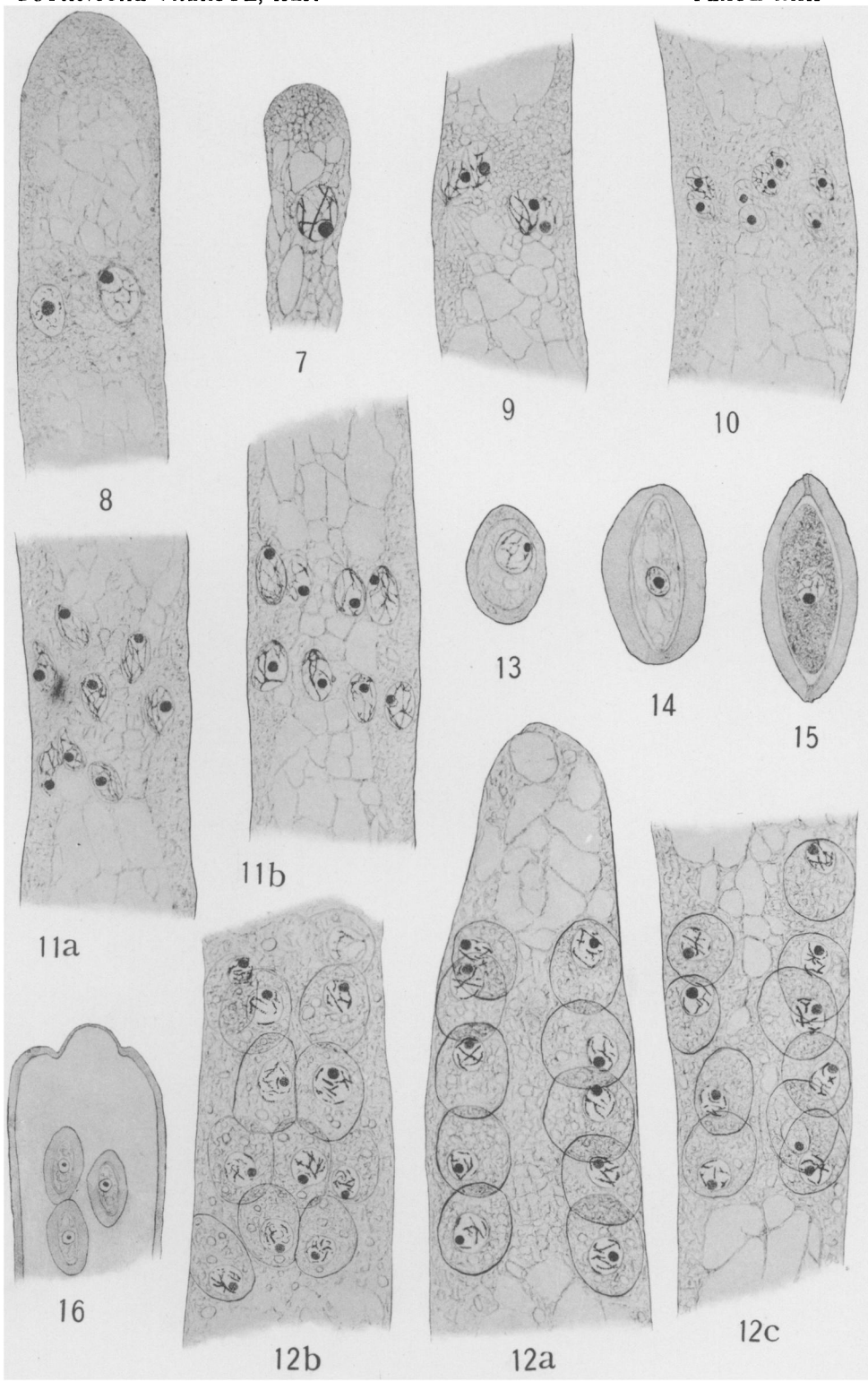
FIG. 14. Still older spore showing spore wall farther advanced.

FIG. 15. Mature spore showing spore wall, terminal pores, cytoplasm, and nucleus.

FIG. 16. Terminal portion of a nearly mature ascus showing operculum.



OVERTON on THECOTHEUS



OVERTON on THECOTHEUS